Histology and Histopathology

Title page

Polyglucosan Bodies in the Prostatic Stromal Smooth Muscles of Aged Dogs

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Short running title: PGB in the Canine Prostates

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Summary

Polyglucosan bodies (PGB) in the prostate of aged dogs without neurological signs were examined by light microscopy, histochemistry and immunohistochemistry. Prostatic PGB were round or oval and slightly basophilic. Most of the bodies were situated within the stromal smooth muscle cells. PGB were intensely positive for PAS, Best's carmine, Lugol's iodine and Grocott's methenamine silver method. Moreover, canine prostatic PGB were immunoreactive for monoclonal antibodies raised against human polyglucosan. The frequency of PGB in the smooth muscle cells was significantly correlated with the age of dogs. The occurrence of PGB in the canine prostate might be a non-specific finding related to ageing.

Keywords: ageing; polyglucosan bodies; prostate; smooth muscle cells
Introduction

Polyglucosan bodies (PGB), the generic name referring to Lafora bodies, corpora amylacea and other similar structures, are composed of glucose polymers (polyglucosan, PG) and, in humans, are observed in the nervous system in ageing and in different pathological conditions (Robitaille et al., 1980; Gray et al., 1988; Cavanagh, 1999; Delgado-Escueta, 2007; Pirici and Margaritescu, 2014). PGB have also been described in extraneuronal tissues (Suzuki et al., 1971; Peress et al., 1979; Cavanagh, 1999; Knowles et al., 2003). In addition to these human cases, PGB have been reported in the intra- and extraneuronal tissues of several animal species with or without neurological disease (Hegreberg and Padgett, 1976; Suzuki et al., 1980; Kamiya et al., 1983, 1991; Cavanagh, 1999; Schoeman et al., 2002; Lohi et al., 2005; Márqueza et al., 2010).

There have been many reports on PGB in several extraneuronal tissues, but only a few in the prostatic stromal smooth muscle, and these only in humans (Suzuki et al., 1971; Dikov et al., 2010).

During a study of ageing phenomena in animals, the present investigators have noticed that numerous PGB are found in the prostatic stromal smooth muscle cells of aged dogs without any neurological signs. The present report provides the morphological features, histochemical characteristics, immunohistochemistry and
Materials and Methods

Twenty six prostate glands were selected randomly from the autopsy profiles of Nippon Veterinary and Life Science University (Table 1). They were aged 2 months to 16 years and 7 months and comprised a wide variety of conditions but had no clinical history of neurological disorder before death. The prostates were fixed in 10% buffered neutral formalin. For the histological examinations, tissue blocks were prepared, embedded in paraffin wax, and sectioned at 5µm. These sections were routinely stained with haematoxylin and eosin (HE) and periodic acid-Schiff (PAS).

For histochemistry, in addition to PAS reaction, Best's carmine, Lugol's iodine and Grocott's methenamine silver method were employed for the detection of PGB (Robitaille et al., 1980; Gray et al., 1988; Cavanagh, 1999).

For immunohistochemistry, mouse monoclonal antibodies (mAB) against human PG (1:100; Kyowa Medex, Tokyo, Japan) and human α-smooth muscle actin (1:400; DAKO, Glostrup, Denmark) were used. Immunohistochemistry was essentially performed as previously described (Kamiya et al., 1990, 1991). Briefly, after incubation with the first antibody, the epitopes were detected using the Envision system™ (Dako,
Tokyo, Japan), with diaminobenzidine as the colour substrate. Slides were then counterstained with haematoxylin.

To estimate the frequency and incidence of PGB in the prostate, five bundles of the smooth muscle cells were randomly selected, and PG-immunopositive round or oval bodies were counted in all cases. The frequency was defined as the ratio (%) of the number of smooth muscle cells including PG-immunopositive bodies to the total number of smooth muscle cells (nuclei). Spearman's correlation coefficient by rank test was used for analysis between the age and the frequency of PG-immunopositive bodies.

Results

In the prostates of 22 (85%) of 26 dogs, PGB were identified, varying in number with individual cases. PGB, 5-20µm in diameter, were variously basophilic, well-delimited round or oval bodies of homogeneous or concentric shapes. Most of the bodies were situated within smooth muscle cells in the prostatic stroma (Fig. 1), but a few were found in the intercellular space. They usually occurred singly, although occasionally two to four bodies were observed together in one smooth muscle cell. With the exception of nuclear pyknosis or displacement, the muscle cells were otherwise unaltered, and the surrounding tissue lacked signs of reaction to the presence of PGB.
PGB could not be found in the glandular portion of any prostate.

Prostatic PGB were intensely positive for PAS (Fig. 1), Best’s carmine (Fig. 2), Lugol’s iodine (Fig. 2 inset) and Grocott’s methenamine silver method.

In immunohistochemical preparations, prostatic PGB reacted positively to the mAB against human PG (Fig. 3). Stromal smooth muscle cells containing PGB were immunoreactive for the mAB against α-smooth muscle actin (Fig. 4). However, PGB were not immunoreactive for α-smooth muscle actin.

The frequencies of PGB are presented in Table 1 and Fig. 5. As shown in Table 1, there was no specific correlation with disease state of the prostate. The numbers of PGB rose in a statistically significant age-related manner ($r = 0.52; p < 0.05$; Fig. 5).

**Discussion**

There have been many reports of PGB in humans and animals (Hegreberg and Padgett, 1976; Robitaille et al., 1980; Suzuki et al., 1980; Gray et al., 1988; Cavanagh, 1999; Schoeman et al., 2002; Lohi et al., 2005; Delgado-Escueta, 2007; Márqueza et al., 2010; Pirici and Margaritescu, 2014). Human and animal PGB are generally positive PAS, Best's carmine, Lugol’s iodine and Grocott’s methenamine silver method.

Ultrastructural studies have shown that PGB are composed mainly of randomly oriented
branching filaments (Gray et al., 1988; Schoeman et al., 2002; Márqueza et al., 2010; Pirici and Margaritescu, 2014). To the best of our knowledge, however, only Suzuki et al. (1971) and Dikov et al. (2010) have briefly reported the existence of PGB in the prostatic stromal smooth muscles in humans. The present study is the first to clarify the morphology, histochemistry and immunohistochemistry of PGB in canine prostate.

Morphologically and histochemically, the PGB in our study were similar to those in the human and animal brain (Hegreberg and Padgett, 1976; Robitaille et al., 1980; Cavanagh, 1999; Lohi et al., 2005; Márqueza et al., 2010; Pirici and Margaritescu, 2014). Most of the bodies are situated within smooth muscle cells, similar to PGB in the human prostate (Suzuki et al., 1971; Dikov et al., 2010). Immunohistochemically, the prostatic PGB in this study had a similar antigenicity to human PG, as previously reported for canine, feline and fox cases (Kamiya et al., 1990, 1991).

The occurrence of PGB in the prostate of humans has been described in various pathological conditions (Suzuki et al., 1971; Dikov et al., 2010). Moreover, it has been reported that PGB in the human CNS and digestive tract are an age-dependent and non-specific phenomenon (Kamiya et al., 1983, 1991; Cavanagh, 1999; Pirici and Margaritescu, 2014). In the present study, the emphasis is on the age-incidence of the prostatic PG as those reported in the CNS and digestive tract. From the results shown in
Table 1 and Fig. 5, the occurrence of PGB in the canine prostate appears to represent an age-dependent phenomenon.

Although the presence of PGB in normal ageing and in various neurological diseases is now well documented, the precise role of PGB has remained elusive (Cavanagh, 1999; Pirici and Margaritescu, 2014). Previous studies have suggested that PGB in the human brain play a role in cellular ageing and cellular responses to oxidative stress (Kimura et al., 1998), most probably by entrapping and sequestration of toxic products resulting from cellular metabolism during the process of ageing (Cavanagh, 1999). The biogenesis of PGB in the canine prostate, therefore, may relate to physiological stress, such as cellular ageing process.

**Conflict of Interest**

We declare no conflict of interest.
References


Lohi H., Young E.J., Fitzmaurice S.N., Rusbridge C., Chan E.M., Vervoort M., Turnbull


Figure Legends

Fig. 1. Numerous PAS-positive PGB in the stromal smooth muscle cells of canine prostate. PAS ×400.

Fig. 2. Numerous PGB show a positive reaction to Best's carmine. ×400. Inset: PGB showing purple brown to Lugol’s iodine. ×400.

Fig. 3. Many PGB in the prostate stromal smooth muscle cells are immunoreactive for monoclonal antibodies against human PG. Envision and haematoxylin. ×400. Inset: Immunoreactive PGB in the neuropil of medulla oblongata. ×400.

Fig. 4. Numerous smooth muscle cells in the prostate stroma are immunoreactive for monoclonal antibodies against α-smooth muscle actin, while PGB (arrows) are not immunoreactive. Envision and haematoxylin ×400, inset ×600.

Fig. 5. Scattergram of counts of PGB per section of prostate in 26 dogs at various ages. Note the great variation in abundance; a correlation coefficient, $r = 0.519$ ($p<0.05$).
<table>
<thead>
<tr>
<th>Dog</th>
<th>Breed</th>
<th>Age (months)</th>
<th>Histological diagnosis</th>
<th>Prevalence (%) of PGB occurrence number (PGB/smooth muscle cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Miniature duchshund</td>
<td>2</td>
<td>Normal</td>
<td>0 (0/1260)</td>
</tr>
<tr>
<td>2</td>
<td>Shiba</td>
<td>11</td>
<td>Atrophy, fibrosis</td>
<td>0 (0/761)</td>
</tr>
<tr>
<td>3</td>
<td>Beagle</td>
<td>24</td>
<td>Normal</td>
<td>0.11 (1/918)</td>
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<tr>
<td>4</td>
<td>Labrador retriever</td>
<td>30</td>
<td>Atrophy</td>
<td>0 (0/1278)</td>
</tr>
<tr>
<td>5</td>
<td>Pug</td>
<td>41</td>
<td>Normal</td>
<td>1.63 (11/673)</td>
</tr>
<tr>
<td>6</td>
<td>Shih Tzu</td>
<td>53</td>
<td>Normal</td>
<td>7.90 (64/810)</td>
</tr>
<tr>
<td>7</td>
<td>Miniature duchshund</td>
<td>60</td>
<td>Hyperplasia</td>
<td>0.31 (2/635)</td>
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<tr>
<td>8</td>
<td>Italian greyhound</td>
<td>72</td>
<td>Purulent prostatitis</td>
<td>20.77 (168/809)</td>
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<tr>
<td>9</td>
<td>Golden retriever</td>
<td>84</td>
<td>Normal</td>
<td>2.68 (21/784)</td>
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<tr>
<td>10</td>
<td>Beagle</td>
<td>96</td>
<td>Hyperplasia</td>
<td>1.40 (12/859)</td>
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<tr>
<td>11</td>
<td>Beagle</td>
<td>102</td>
<td>Prostatitis</td>
<td>4.21 (25/594)</td>
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<tr>
<td>12</td>
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<td>102</td>
<td>Hyperplasia</td>
<td>3.07 (23/748)</td>
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<tr>
<td>13</td>
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<td>0.50 (6/1205)</td>
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<tr>
<td>15</td>
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<td>120</td>
<td>Malignant melanoma (metastasis)</td>
<td>0.35 (2/569)</td>
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<td>16</td>
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<td>124</td>
<td>Atrophy</td>
<td>4.32 (58/1344)</td>
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<td>17</td>
<td>Beagle</td>
<td>128</td>
<td>Atrophy</td>
<td>18.33 (184/1004)</td>
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<td>18</td>
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<tr>
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<td>Hyperplasia</td>
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<tr>
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<td>168</td>
<td>Normal</td>
<td>12.30 (102/829)</td>
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<tr>
<td>24</td>
<td>Brittany spaniel</td>
<td>180</td>
<td>Hypertrophy</td>
<td>2.40 (28/1169)</td>
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<td>25</td>
<td>Labrador retriever</td>
<td>183</td>
<td>Normal</td>
<td>34.84 (487/1398)</td>
</tr>
<tr>
<td>26</td>
<td>Pug</td>
<td>199</td>
<td>Hyperplasia</td>
<td>11.00 (170/1545)</td>
</tr>
</tbody>
</table>
A graph showing the correlation between age in months and the percentage of PGB occurrence. The correlation coefficient is $r = 0.519$ with $p < 0.05$.